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Serological screening test for infectious mononucleosis using papain-treated sheep erythrocytes

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SYNOPSIS A screening test for infectious mononucleosis is described, based on the fact that antibodies against sheep red cells remaining after absorption of the serum by papain-treated sheep red cells appear to be specific for the disease. The test appears to be more useful in diagnosis than classical absorption techniques and has the advantage of being specific while maintaining a high degree of sensitivity.

The classical absorption and agglutination techniques for the serological diagnosis of infectious mononucleosis (Davidsohn, 1937) give well-defined results in most cases, but the results for low titre sera may be difficult to interpret. The antigen on sheep erythrocytes which reacts with sera from cases of glandular fever is inactivated or removed by treatment of the cells with papain (Wöllner, 1955; Muschel and Piper, 1959), the virus receptor destroying enzyme of cholera vibrio, influenza virus, and proteolytic enzymes of vegetable origin (Springer and Rapaport, 1957). This property forms the basis of serological tests for glandular fever which give more clear-cut results than classical absorption tests (Muschel and Piper, 1959).

The present study compares the results obtained by the classical absorption and enzyme techniques, with special reference to low titre sera. The superiority of the enzyme method is confirmed, and a screening test is proposed, based on the fact that antibody to sheep red cells that remains after absorption of the serum by papain-treated cells appears to be specific for infectious mononucleosis.

METHODS

PREPARATION OF PAPAIN SOLUTION (ALBREY AND SIMMONS, 1960) Add sufficient M/15 disodium phosphate solution in distilled water to 100 ml. M/15 monosodium phosphate solution to raise the *p*H to 5·8 to 6·0. Mix 2 g. papain powder (Merck or B.D.H.) in a mortar with a small amount of buffer to form a paste. Dilute the paste in a stoppered container to 100 ml. with the phosphate buffer and stand at room temperature for one to two hours. Centrifuge, draw off the cloudy supernatant and Seitz filter to clear solution, using excess buffer to wash the filter before and after filtration. Add 5 ml. 1M cysteine hydrochloride solution and make up to 200 ml. with more buffer. Adjust to a final *p*H of 2·8 to 3·0 with 1N HCl or 1N NaOH if necessary. Incubate at 37°C. for one hour, and dispense in amounts of 2 to 5 ml. Store in deep freeze at −20°C. The solution will keep in the deep freeze for at least two months, and probably for much longer. Small amounts of thawed material may be kept at 4 to 6°C. for two to three days, but the solution should not be re-frozen after thawing.

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MODIFIED ROUS-TURNER SOLUTION (SIMMONS, GRAYDON, SEMPLE, AND TAYLOR, 1951) Glucose, (34·56 g.), dissolved in 640 ml. distilled water (5·4% solution) and 10·03 g. tri-sodium citrate (Merck) dissolved in 264 ml. distilled water (3·8%) are mixed and filtered through sintered glass 5/3. The final *p*H should be approximately 7·4.

TREATMENT OF ERYTHROCYTES Sheep erythrocytes are washed three times with 0·9% saline. The enzyme solution is diluted with twice its volume of modified Rous-Turner solution; 1 volume of packed cells is suspended in 3 volumes of diluted papain. The mixture is incubated in 37°C. for 15 minutes, and the enzyme-treated cells then washed three times with saline before packing for the test.

ABSORPTION OF SERUM WITH ENZYME-TREATED CELLS One volume of inactivated serum (56°C. for 30 minutes) is diluted with 4 volumes of saline and added to 2 volumes of packed papain-treated cells. After being mixed thoroughly, the suspension is allowed to stand at room temperature for one hour. The mixture is then centrifuged to give a supernatant of absorbed serum diluted one in five. A single absorption appears to be sufficient.

CLASSICAL DIFFERENTIAL ABSORPTIONS Absorptions with guinea-pig kidney and ox cells were carried out by the methods described by Dacie and Lewis (1963).

TITRATIONS Doubling dilutions of the 1:5 diluted serum are made in glass concavity tiles; an equal volume of a 2% suspension of sheep cells in saline is added to each dilution and the tiles left at room temperature for two hours. They are then gently rocked and readings made

macroscopically over diffuse light. Titres are expressed as the reciprocal of the highest dilution at which agglutination is still detectable.

SCREENING TEST One drop of serum absorbed with papain-treated sheep cells is placed on a glass tile and one drop of 2% untreated sheep cells added. Serum and cells are mixed and allowed to stand at room temperature for 30 minutes. The tile is then gently rocked and agglutination which is macroscopically evident taken as a positive result. A titration may be made for follow-up purposes.

INTERPRETATION OF RESULTS

The criteria of positive results are as follows. The references to titre are all set out in Table I.

CLASSICAL ABSORPTION TESTS (a) Guinea-pig kidney absorption: not more than a three tube reduction in titre of unabsorbed serum (titre 1 in Table I) after absorption with guinea-pig kidney (titre 5).

(b) Ox cell absorption: at least a four tube decrease in titre of unabsorbed serum after absorption with ox cells (6).

ENZYME TESTS (a) 'Wöllner test': reduction in titre against papain-treated cells (2) from titre against normal cells (1)

(b) 'Wöllner test 2': titre of absorbed serum against enzyme-treated cells (4) at least four tubes lower than titre against untreated cells (3); the fact that the titres in column 4 did not fall to zero after a single absorption is probably due to the persistence of non-specific antibody against receptors on the red cell exposed by papain treatment; after repeated absorption of the serum by papain-treated cells (titre in col. 4 < 10), the titre for untreated cells (3) remained the same as before.

SCREENING TEST Agglutination of untreated sheep erythrocytes by serum absorbed with papain-treated erythrocytes (titre 3).

NEGATIVE AND DOUBTFUL RESULTS Tests were recorded as negative when the criteria above were not fulfilled, and as 'doubtful' when borderline results were obtained (Tables IV and V).

RESULTS

Samples of sera from 300 patients for whom a diagnosis of infectious mononucleosis was considered likely on clinical examination were fully investigated (Table II).

Seventy-two of the patients (Table III) showed a

TABLE I

TYPICAL POSITIVE SEROLOGICAL TESTS FOR INFECTIOUS MONONUCLEOSIS

Patient No.	*Titre of Unabsorbed Serum against*		*Titre of Serum Absorbed with Papainized Cells against*		*Titre against Untreated Cells of Serum Absorbed with*	
	Untreated Cells (1)	*Papainized Cells* (2)	*Untreated Cells* (3)	*Papainized Cells* (4)	*Guinea-pig Kidney* (5)	*Ox Cells* (6)
69	320	160	320	< 10	160	< 10
77	640	160	640	10	640	10
252	80	40	80	< 10	80	< 10

TABLE II

RESULTS OF SEROLOGICAL INVESTIGATION OF PATIENTS CLINICALLY SUSPECTED OF HAVING INFECTIOUS MONONUCLEOSIS

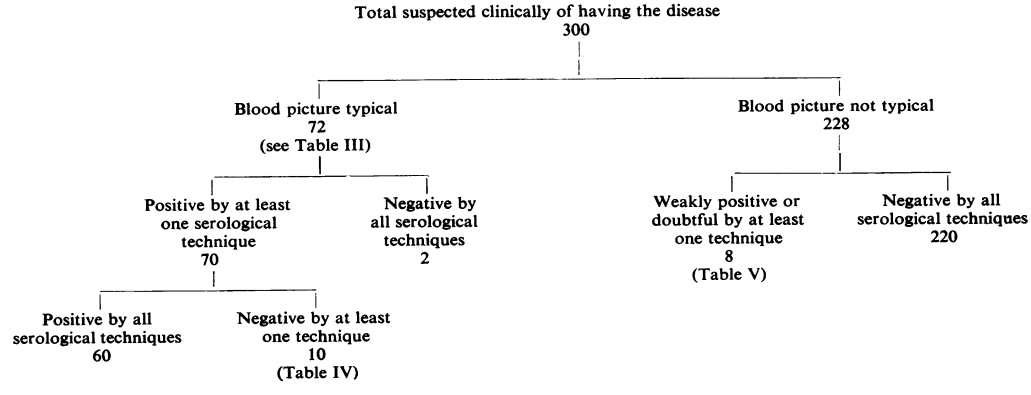

TABLE III

SEROLOGICAL TESTS ON THE 72 PATIENTS WITH BLOOD PICTURE TYPICAL OF GLANDULAR FEVER

	Positive	*Negative or Doubtful*
Classical absorptions		
(a) Guinea-pig kidney	70	2
(b) Ox cell	65	7
both (a) and (b) positive	65	7
Enzyme tests		
'Wöllner test 1'	62	10
'Wöllner test 2'	63	9
Screening test	70	2

blood picture considered typical of the disease, *i.e.*, a relative and absolute lymphocytosis with a significant number (at least 15% of the total, but usually a majority) of so-called atypical forms. Of these, 60 gave typical positive results by all serological techniques whilst two gave negative findings by all techniques. The remaining 10 gave negative findings by at least one serological test (Table IV). Of these 10, all gave a positive guinea-pig absorption test; five were positive by ox cell absorption, *i.e.*, five positive by Davidsohn's criteria of a positive classical absorption test; two were positive by 'Wöllner test 1', three by 'Wöllner test 2', and all gave a positive screening test.

A weakly positive or doubtful classical absorption test (guinea-pig, or ox cell or both) was given by sera from eight patients clinically suspected of having glandular fever, but without blood findings typical of the condition (Tables II and V). A small number of atypical lymphocytes was seen in films from some of these cases. One was subsequently shown to have osteomyelitis, and four to have infective hepatitis. The diagnosis in the remainder could not be determined. None of these sera gave a positive screening test (col. 3, Table V).

As controls, sera from 2,000 subjects who were normal or were not suspected clinically of having glandular fever were subjected to the papain screening test. All gave a negative reaction except three. These were subsequently found to be positive by all techniques, to have a blood film suggestive of infectious mononucleosis, and clinical features consistent with this diagnosis.

DISCUSSION

It would appear that antibody to sheep red cells remaining after absorption of serum with papain-

TABLE IV

SEROLOGICAL TESTS ON SERA FROM THE 10 PATIENTS WITH TYPICAL BLOOD PICTURES BUT NEGATIVE TO AT LEAST ONE SEROLOGICAL TEST FOR INFECTIOUS MONONUCLEOSIS

Patient No.	*Titre Unabsorbed Serum against*		*Titre of Serum Absorbed with Papainized Cells against*		*Titre against Untreated Cells of Serum Absorbed with*	
	Untreated Cells (1)	*Papainized Cells* (2)	*Untreated Cells* (3)	*Papainized Cells* (4)	*Guinea-pig Kidney* (5)	*Ox Cells* (6)
81	40	40	20	< 10	20	< 10
121	40	20	40	< 10	20	< 10
132	40	40	40	10	20	< 10
140	80	40	80	< 10	20	< 10
160	40	40	40	< 10	20	< 10
54	80	640	40	10	40	40
161	20	160	20	10	20	< 10
230	40	160	20	10	20	40
285	20	320	20	10	20	< 10
288	20	80	10	< 10	10	< 10

TABLE V

SEROLOGICAL TESTS ON SERA FROM THE EIGHT PATIENTS WITH WEAKLY POSITIVE OR DOUBTFUL CLASSICAL ABSORPTION TEST

Patient No.	*Titre Unabsorbed Serum against*		*Titre of Serum Absorbed with Papainized Cells against*		*Titre against Untreated Cells of Serum Absorbed with*	
	Untreated Cells (1)	*Papainized Cells* (2)	*Untreated Cells* (3)	*Papainized Cells* (4)	*Guinea-pig Kidney* (5)	*Ox Cells* (6)
6	40	320	< 10	20	10	< 10
13	80	640	< 10	< 10	20	< 10
25	10	20	< 10	< 10	10	< 10
45	40	160	< 10	10	20	10
49	40	320	< 10	10	10	10
58	10	160	< 10	< 10	10	10
274	20	80	< 10	10	20	20
283	20	320	< 10	20	10	10

treated sheep erythrocytes is a more reliable indication of infectious mononucleosis than other enzyme tests or classical absorption procedures.

The lower agglutinating activity of unabsorbed serum against papain-treated cells compared with untreated cells is the basis of a rapid slide test for the disease (Lovric, 1961, 1965). However, the inconsistency of this difference in glandular fever (Davidsohn and Lee, 1964), and the fact that low titre sera may not react with untreated sheep cells in the time specified by the test cause false negative results. False positive results also may occur with this test.

The classical absorption test is well known to give a significant proportion of results which are difficult to interpret and whose significance is often no clearer when the test is repeated or carried out on serum taken several weeks later. Titres for the papain screening test were close to those obtained after guinea-pig absorption (compare columns 3 and 5 in Tables I and IV). However, when titres for the differential absorption test were doubtful (see Tables IV and V), *e.g.*, early in the disease, in convalescence, or as an individual characteristic, or when they were falsely positive, the enzyme test was unequivocal. The screening test gave no false positive reactions and the incidence of false negatives appeared to be lower than with other techniques.

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